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Review Article

Induced pluripotent stem cell technology for dissecting the cancer epigenome

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Cancer arises through the accumulation of both genetic and epigenetic alterations. Although the causal role of genetic mutations on cancer development has been established *in vivo*, similar evidence for epigenetic alterations is limited. Moreover, mutual interactions between genetic mutations and epigenetic alterations remain unclear. Cellular reprogramming technology can be used to actively modify the epigenome without affecting the underlying genomic sequences. Here we introduce recent studies that have utilized this property for cancer research. We propose that just as it has potential for regenerative medicine and disease modeling, cell reprogramming could also be a powerful tool for dissecting the role of the cancer epigenome in the development and maintenance of cancer cells.

Cancer arises through the accumulation of multiple genetic alterations, suggesting it is a genetic disease.[1] However, many studies have revealed that the initiation and promotion of cancer development are accompanied by various epigenetic alterations, which are often characterized by epigenetic modifications that differ from those in normal cells.[2–4] For instance, overall DNA methylation levels are reduced in most cancer cells,[5] while site-specific DNA hypermethyaltion at gene promoters[6] is one of the most extensively analyzed alterations in cancer cells.[7] A role of an altered epigenome in cancer development has been demonstrated previously. For example, reduced DNA methylation increases chromosomal instability,[8,9] leading to increased tumor frequency *in vivo*.[10,11] In contrast, DNA hypermethylation is mainly associated with the silencing of tumor suppressor genes, such as *p16ink4A*,[12,13] *VHL*,[14] *BRCA1*[15] and *LKB1*.[16] Indeed, a suppressive effect through the forced reduction of DNA methylation on cancer development has been shown in several cancer models.[17–23] However, the mechanisms driving these epigenetic changes during carcinogenesis are poorly understood.

Recent genome-wide sequencing studies have revealed that epigenetic modifier genes are often mutated in many types of cancers.[24–26] Both activating[27] and inactivating[28,29] mutations of *Ezh2*, which encodes a catalytic subunit of polycomb repressive complex 2 (PRC2), have been identified in certain types of cancers. *Tet2*, which is associated with 5hmC production and DNA demethylation, is also mutated in myeloid malignancies and other hematological disorders.[30] These findings strongly suggest that genetic abnormalities are the primary cause of the altered epigenome in cancer cells.

It should also be noted that epigenetic modifications are tightly coupled with transcriptional regulations. Generally, oncogenic signals affect the transcriptional regulation of downstream target genes, thereby resulting in altered transcriptional networks. Given that oncogenic signals are often activated by genetic mutations, altered epigenetic patterns could be a secondary effect of the transcriptional changes caused by the genetic alterations.

In addition, epigenetics regulates the adaptation of transcriptional networks to the extracellular environment, such as nutrition levels, oxygen concentration and inflammation. Thus, for some cancers, it has been proposed that such environmental factors could induce epigenetic alterations. In particular, inflammation is considered a key environmental factor for both cancer development and epigenetic modifications in cancer cells. Dextran sodium sulfate (DSS), an inflammation inducer that is also a potent tumor promoter in the colon, causes abnormal hypermethylation at DNA methylation valleys in

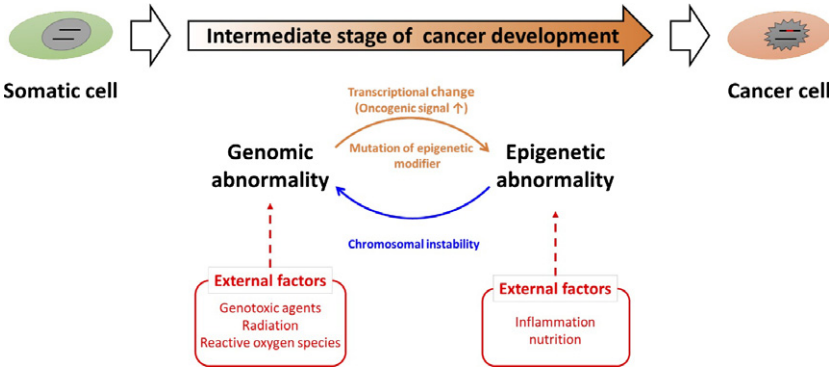

Fig. 1. Crosstalk between genomic and epigenetic abnormalities during cancer development. Cancer arises from a somatic cell accumulating genetic and epigenetic abnormalities. During its progression, external factors cause additional genetic and epigenetic changes in the cell. The genetic abnormalities can regulate the epigenetics via alterations in transcriptional networks. In contrast, the epigenetic abnormalities can regulate genomic integrity through chromosomal instabilities.

murine colonic crypts.[31] Similarly, *Helicobacter pylori* infection, which is closely associated with gastric cancer development in both humans and rodents, causes abnormal DNA methylation in normal gastric mucosa.[32,33] Notably, a subset of epigenetic alterations caused by such inflammation are consistent with those in cancer cells, suggesting that environmental factors may contribute to cancer development by inducing epigenetic alterations.[34] Considering that these environmental factors are independent of any genetic abnormalities in cancer cells, inflammation-dependent epigenetic alterations could be an independent cancer driver of genetic abnormalities.

All together, both genetic mutations and environmental factors can induce epigenetic alterations in cancer cells. Given that the correction of genetic mutations is not a feasible cancer therapy, epigenetic alterations caused by environmental factors may make better targets. However, it remains unclear to what extent environmental factors inducing epigenetic modifications play in cancer development (Fig. 1).

The study of this relationship may benefit tremendously from cell reprogramming methods, of which there are three. In the first method, nuclear transplantation, the nucleus of a somatic cell is transferred into an enucleated oocyte.[35] In the second method, cell fusion, a somatic cell is fused with an embryonic stem cell (ESC).[36] Finally, the third method, induced pluripotent stem cells (iPSC), describes somatic cells that have been converted to a pluripotent state by the transient induction of reprogramming factors (*Oct3/4*, *Sox2*, *Klf4* and *cMyc*).[37,38] Along with pluripotency, iPSC exhibit unlimited growth capacity and, therefore, can be a promising cell source for regenerative medicine, disease modeling and drug discovery.[39–43] It has been shown that dynamic changes of the epigenome occur during the reprogramming, whereas changes in the underlying genomic sequences are not required.[44,45] Therefore, reprogramming technology could be a useful strategy for dissecting the role of genetic mutations and epigenetic alterations in cancer cells.

Cancer Cell Reprogramming

To study the role of epigenetic alterations in cancer development, one promising strategy would be to investigate the phenotypic consequence of a manipulation of the cancer epigenome. Because epigenetic regulations play a pivotal role in cell fate determination and their maintenance, the cell fate control by reprogramming technology should be accompanied by dynamic changes of the epigenome. Hochedlinger *et al.* [46] report the reprogramming of the cancer nucleus by nuclear transplantation. Notably, the oocytes that received the cancer nuclei were subsequently able to form a blastocyst-like structure. The authors further succeeded to establish nuclear transferred ESC (ntESC) with the melanoma nucleus of tetracycline-inducible *HRAS* transgenic mice,[46] demonstrating that the cancer genome is reprogrammable into the pluripotent stem cell state. However, ntESC could not be generated when using nuclei from other types of cancer cells, such as leukemia, lymphoma and breast cancer cells. This inability suggests that cancer cells exhibit refractoriness to nuclear reprogramming. Notably, there are several reports that have succeeded to establish iPSC from cancer cells. These studies have revealed interesting insights of the cancer epigenome with regards to the lineage specificity of oncogenes,[47,48] recapitulation of cancer progression[49] and cancer cell heterogeneity (Fig. 2).[50]

Lineage specification of oncogenes. Specific cancers are often associated with mutations at specific genes.[51] For example, *HER2* amplification is preferentially observed in breast cancers,[52] and *EGFR* mutation is frequently detectable in lung cancers.[53,54] Patients with familiar adenomatous polyposis (FAP) show a mutation in the *APC* gene and develop cancers predominantly in the colon, although cells throughout the patient body will harbor mutations in this gene.[55] These observations suggest that the genetic mutations require the specific cell type to exert cancer properties.

Carette *et al.*[47] established iPSC from human chronic myeloid leukemia (CML) cells that harbor the *BCR-ABL* fusion gene. CML-derived iPSC lost sensitivity to tyrosine kinase inhibitors (TKI) that targeted BCR-ABL despite expression of the *BCR-ABL* gene. Intriguingly, the TKI sensitivity was recovered when CML-iPSC were differentiated into hematopoietic cell lineage cells.[47] Given that the iPSC and hematopoietic lineage cells share the same genetic context, these results indicate that TKI sensitivity depended on the differentiation status of cells with distinct epigenetic regulation.

Similarly, Stricker *et al.*[48] established glioblastoma (GBM)-derived iPSC from GBM-derived neural stem cells. Re-differentiation of the GBM-iPSC into neural progenitors resulted in highly malignant cells when injected into immunocompromised mice. However, GBM-iPSC did not exhibit the malignant phenotype when differentiated into non-neural lineage cells. These results too suggest that genetic mutations render the cells malignant only when a particular cell type with the unique epigenetic state is met.[48]

Recapitulation of human cancer progression. *In vivo* cancer models are often used to study the molecular mechanisms for the cancer initiation and progression, but species differences between humans and rodents have compromised the development of effective cancer therapies and the recapitulation of oncogenesis in human cancer cells. For this reason, iPSC may make a better model.

Kim *et al.*[49] succeeded in establishing iPSC from human pancreatic ductal adenocarcinomas (PDAC) that can be main-

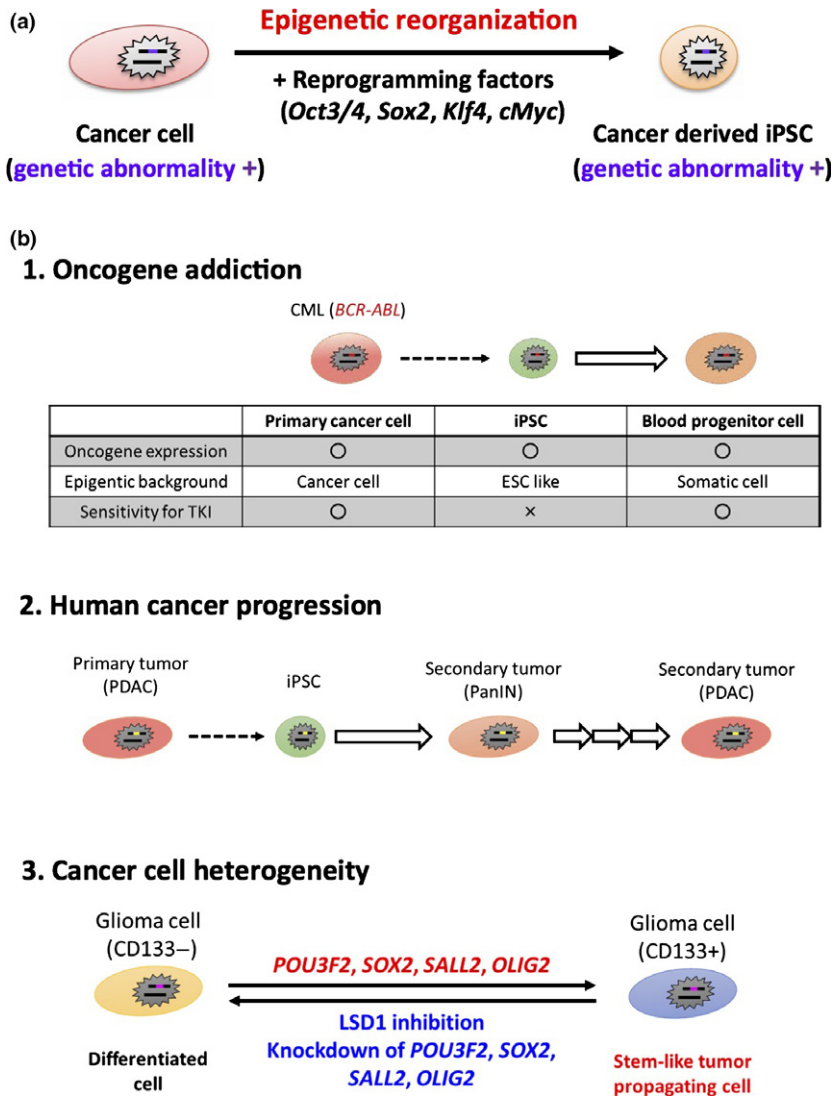

Fig. 2. Application of cell reprogramming to cancer biology. (a) Cell reprogramming can alter the epigenome of a cancer without affecting genetic abnormalities. (b) Cell reprogramming as a tool for dissecting the unique properties of cancer cells. (1) Reprogramming of chronic myeloid leukemia (CML) cells showed epigenetic background-specific oncogenic addiction. (2) Re-differentiation of cancer cell-derived iPSC can recapitulate the progression of human cancer development. PanIN, pancreatic intraepithelial neoplasia; PDAC, pancreatic ductal adenocarcinoma. (3) Cancer cell heterogeneity can be controlled by the reprogramming of cancer cells.

tained under the low level expression of exogenous 4Fs. Upon the induction of differentiation, the PDAC-iPSC differentiated into three germ cell layers and formed teratomas, reflecting their pluripotency. Interestingly, these PDAC-iPSC-derived teratomas contributed to endodermal ductal structures, which exhibited a similar histology to pancreatic intraepithelial neoplasia (PanIN), a premalignant lesion of PDAC. The authors proposed that PDAC-iPSC can recapitulate the early stage of pancreatic cancer development upon their differentiation, and that this disease model could be useful for studying the progression of human pancreatic cancer cells. Furthermore, it was shown that the HNF4a is involved in the pancreatic cancer progression and invasion, and, thus, could be a therapeutic target. Taken together, multipotent cancer cells established by reprogramming technology have provided a useful platform for studying human cancer genome–epigenome interactions and discovering key molecules in cancer progression.

Hierarchy of heterogeneous cancer cells. Cancer cells are notoriously heterogeneous, a property that can be attributed to their genetic and epigenetic variations.[56] It has been suggested that heterogeneity could be a driving force in cancer progression and is a fundamental basis of the cancer stem cells (CSC) concept.[57] In this concept, a CSC population resides atop the hierarchy and has the potential to give rise to heterogeneous cancer cells and reconstitute tumor mass. However, the hierarchy of heterogeneous cancer cells is not fully understood. Suvà *et al.*[50] report that differentiated glioma cells can be converted into CSC-like populations by the forced expression of a specific set of transcription factors (*POU3F2*, *SOX2*, *SALL2* and *OLIG2*), which are highly expressed in stem-like glioma cells. The forced expression of these factors in non-CSC-like glioma cells reorganized the transcription network and epigenetic landscape into a CSC-like state. Furthermore, the authors also identified LSD1 and RCOR1, which mediate the demethylation of H3K4, as an epigenetic switch for the conversion of non-CSC-like glioma cells into CSC-like glioma cells. Consistently, they showed that an LSD1 inhibitor induced the ablation of stem-like glioma cells and that *LSD1* knockdown have prolonged survival time and reduced tumorigenic potential *in vivo*. These findings support the concept that control of CSC-like glioma cells and differentiated glioma cells is governed by epigenetic regulation. Similarly, a recent study demonstrated that biological interconversion between glioma stem-like cells and differentiated glioma cells is reversible and functionally plastic.[58] Notably, this interconversion is accompanied by gain or loss of PRC2-mediated H3K27me3

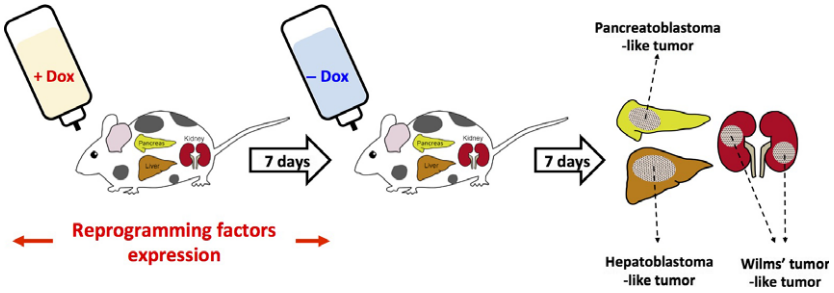

Fig. 3. Generation of human pediatric cancer-like tumors *in vivo*. An *in vivo* reprogramming system was established with Dox-inducible alleles for reprogramming factors. Premature termination of *in vivo* reprogramming in mice causes cancer development consistent with human pediatric cancers (Wilm's tumor-like tumor in the kidney, hepatoblastoma-like tumor in the liver and pancreatoblastoma-like tumor in the pancreas).

on developmental genes. All together, these results suggest that hierarchical control of heterogeneous cancer cells could be bidirectional and that such interconversion could be a promising target for efficient cancer treatment.

Induction of Dedifferentiation *In Vivo* Using Reprogramming Technology

In vivo reprogramming systems have provided a unique platform for studying the role of dedifferentiation in cancer development and provided the first *in vivo* evidence that epigenetic abnormalities can be a driving force for cancer development.[59]

Reprogramming systems *in vivo*. Stadtfeld *et al.*[60] generated mice containing lentivirus-mediated transgenic alleles for doxycycline (Dox) inducible reprogramming factors. The transgenic mice often developed teratomas consisting of differentiated cells of three different germ layers even without Dox treatment, presumably because of the leaky expression of 4Fs. Considering that pluripotent stem cells are capable of teratoma formation, this observation strongly suggested that somatic cells can be reprogrammed into pluripotent stem cells *in vivo*. In a later study, Abad *et al.*[61] established germline-transmitted mice with lentivirus-mediated Dox-inducible reprogrammable alleles and found that these mice form teratomas in response to Dox treatment, again suggesting *in vivo* reprogramming. Notably, these mice formed various types of tumors (Wilms' tumor, skin papilloma, urothelial carcinoma and intestinal polyps) as well as teratomas upon Dox treatment of various periods.

Ohnishi *et al.*[59] also established another reprogrammable mouse model in which the expression of reprogramming factors can be controlled by Dox and visualized by the fluorescent protein mCherry.[62] In this system, the induction of reprogramming factors for 28 days resulted in multiple formations of teratomas in various organs. Importantly, *in vitro* culture of teratoma tissue led to the derivation of iPSC that could be used for the generation of adult chimeric mice, demonstrating that somatic cells are reprogrammable *in vivo* with this system. All together, these results showed that the expression of reprogramming factors *in vivo* can alter the cellular identity of adult somatic cells into the pluripotent state in living mice.

Premature termination of *in vivo* reprogramming. In reprogrammable mice, the long-term expression of 4Fs resulted in the establishment of pluripotent stem cells. Interestingly, the *in vivo* phenotype varied with the length of time of the 4F expression. A short-term induction of 4Fs caused the emergence of dysplastic cancer-like cells exhibiting abnormal proliferation. However, the dysplastic cells disappeared after withdrawal of 4F expression and reverted to normal-looking cells. The reversion of the dysplastic phenotype indicates that continuous expression of 4Fs is required for the maintenance of the dysplastic cells and further suggests that early dysplastic cells retain epigenetic memory. However, prolonging the induction of the reprogramming factors to a period that remains shorter than that required for teratoma development caused 4F-independent tumor formation in various organs. These tumors consisted of dysplastic cells that were distinct from teratoma cells. Moreover, the dysplastic cells had an invasion phenotype and in particular cases metastasized into the lymph node, suggesting that they behave like cancer cells. Furthermore, the late dysplastic cells exhibited activation of ESC-Core and ESC-Myc modules, indicating that acquisition of the transcription network of pluripotent stem cells is associated with the tumor development.[63]

Interestingly, 4F-independent tumors resemble human pediatric cancers, such as Wilms' tumor-like tumor in the kidney, hepatoblasotoma-like tumors in the liver and pancreatoblastoma-like tumors in the pancreas. Wilms' tumor is the most common pediatric kidney cancer (Fig. 3). Although genetic mutations, such as *WT1* and *WTX*, have been identified in Wilms' tumors, the overall incidence of these mutations is not high.[64] In contrast, it has been shown that abnormal DNA methylation patterns at imprinting loci are frequently detectable in Wilms' tumors.[65,66] Of note, the failed reprogram-

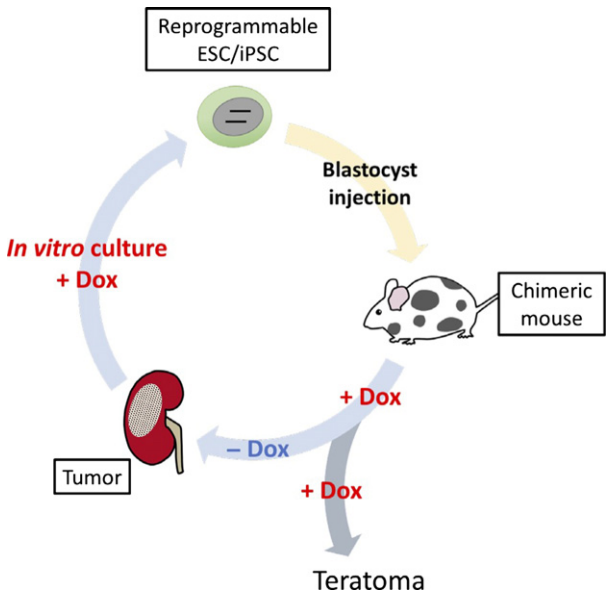

Fig. 4. Proof of concept for epigenetics-driven cancer development. *In vivo* reprogramming results in teratoma formation, whereas premature termination of the reprogramming leads to pediatric cancer-like tumors. The tumor cells can be reprogrammed into iPSC that can be used to generate adult chimeric mice. Note that tumor-derived iPSC in this model give rise to non-neoplastic cells in the chimeric mice.

ming-associated tumors lacked detectable genetic mutations in cancer-related genes, but often exhibited a biallelic expression of imprinting genes, which was accompanied by abnormal DNA methylation patterns.[59] These observations suggest that epigenetic alterations related to the cellular reprogramming might be involved in the development of a subset of pediatric cancers.

Proof of concept for epigenetics-driven cancer development *in vivo*. Considering that somatic cell reprogramming does not require particular genetic alterations, but rather a reorganization of the epigenome, cancer development through the transient expression of reprogramming factors raises the possibility that epigenetic alterations drive carcinogenesis. Notably, upon the re-induction of reprogramming factors, failed reprogramming-associated cancer cells were reprogrammed into iPSC with higher efficiency and shorter latency, presumably reflecting the partial reprogramming state of these cells. Of particular note, kidney cancer-derived iPSC were able to give rise to non-neoplastic kidney cells after injection into blastocyst. Given that iPSC derivation and differentiation do not require genetic mutations, the contribution of cancer-iPSC to non-neoplastic kidney has provided the first experimental evidence for epigenetics-driven cancer development *in vivo* (Fig. 4).[67,68] However, considering that endogenous *Oct3/4* is deeply silenced in somatic cells,[69] it is unlikely that expression of Yamanaka 4 factors is directly involved in the development of human cancers. It should be noted that somatic cell reprogramming can be achievable with other sets of transcription factors, which do not include Yamanaka 4 factors.[70,71] In this context, it would be of great interest to identify environmental factors that induce such transcription factor expression, which might eventually cause cellular reprogramming. Further analyses using human samples are needed to clarify the role of reprogramming in human cancer development.

Conclusion

Induced pluripotent stem cell technology has already gained strong interest for its potential in regenerative medicine and disease modeling. It is becoming increasingly clear that this technology can also advance cancer research by uncovering the role of epigenetic alterations in cancer development and cancer cell maintenance. Given that the epigenome can be modified with small chemical compounds, understanding its role should contribute to effective cancer-treatment strategies.

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Disclosure Statement

The authors have no conflict of interest to declare.

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